

Primary Reviewer: John Doherty
ReRegistration Branch III, HED
Secondary Reviewer: William Sette
Toxicology Branch, HED

TXR # 0050380.

Study Type: Acute Neurotoxicity - rat (870.6200, or 81-8)

PC Code: 074801
DP Barcode: D270929
Submission No.: S586450

Test Material: Technical tribufos (DEF), S,S,S-tributyl phosphorotrithioate (98.4 to 98.5% purity).

Citation: L.P. Sheets and R.G. Gilmore (and B.P. Stuart), 2000, "An acute oral neurotoxicity study with technical grade tribufos (DEF®) in Wistar rats" Bayer Corporation, Agricultural Division, Stilwell, Ks, Study No.: 98-412-WF, July 31, 2000. MRID No.: 45194401.

In-Life Phase: January 18, 1999 to February 5, 1999.

Executive Summary:

In this acute neurotoxicity study (2000, MRID No.: 45194401), four groups of 18/sex Wistar strain rats were dosed as control, 2, 20 or 100 mg/kg of tribufos (98% purity in corn oil at 5 mL/kg). Twelve rats/sex per group were assessed for clinical signs, body weight, FOB and motor activity at pretest, estimated time for peak effects (six hours post dosing based on preliminary studies), and on days 7 and 14 postdosing. At termination the 6rats/sex/group were perfused and assessed histologically for neuropathological effects. The remaining 6 rats/sex/group were assessed for cholinesterase at pretest and the time of peak effect.

Systemic Toxicity, FOB and Motor Activity. At 20 mg/kg statistically significant ($p < 0.05$) decreased motor (interval range ~40-81%, total 44%) and locomotor (interval range~38-82%, total 39%)) activity was evident in males. In females statistical significance was not attained but decreases in motor activity (interval range 45 to 68%, total 8%) and in locomotor activity (interval range 51 to 81%, total 14%) were apparent. At 100 mg/kg the decreases in motor and locomotor activity were larger to indicate a dose response and the locomotor activity decrease in females was statistically significant. There was also decreased rearing and arousal. Body temperature was decreased 1.9° C and 2.8° C in males and females respectively. Two deaths among the females on days 2 and 4 were attributed to tribufos. **The LOAEL is 20 mg/kg based on decreased motor activity. The NOAEL is 2 mg/kg.**

Cholinesterase. Plasma ChE data for females were confounded by there being an apparently higher control reading with this group being up to 22% higher in the pretest assessment relative to the other groups. Thus, the 37% (not statistically significant) lower value for the 2 mg/kg dose group could represent 15% actual inhibition. Plasma ChE was statistically significantly ($p < 0.05$) inhibited at 20 mg/kg (64% _ and 92% _) and at 100 mg/kg (85% _ and

98%_) when assessed 6 hours after dosing. RBC AChE was also statistically significantly ($p < 0.05$) inhibited at 20 mg/kg (41% _ and 55% _) and 100 mg/kg (81% _ and 85% _) mg/kg. Brain AChE was statistically significantly inhibited (16%, $p < 0.05$) in the 100 mg/kg female dose group only. **The LOAEL for cholinesterase inhibition is 20 mg/kg based on inhibition of both plasma ChE and RBC AChE. The NOAEL is 2 mg/kg. The NOAEL and LOAEL for brain AChE inhibition is 20 and 100 mg/kg.** The dose level of 2 mg/kg may actually be a threshold level for plasma ChE inhibition since there was potentially 15% inhibition.

Neuropathology. There were no lesions in the nervous system attributed to tribufos treatment.

Classification: This study is classified as ACCEPTABLE/Guideline for a series 870.6200 acute neurotoxicity study.

Compliance: Signed and dated Statements of Data Confidentiality (no claim), GLP and Quality Assurance were provided. There was no flagging statement provided. No flagging criteria were exceeded as judged by the reviewer.

Study Constants and Materials

Test Material

Chemical: Technical grade DEF (tribufos)
Chemical Name: S,S,S-tributyl phosphorotrithioate
CAS No.: 78-48-8
Purity: 98.5% on October 1998 and 98.4% on April 1999
Batch No.: 503-0087
Description: clear colorless liquid

Test Animals

Species: Rat (males and females)
Strain: Wistar (CrI:WI(HAN)BR)
Source: Charles River Laboratories (Raleigh, NC)
Age: 9 weeks at start of treatment.
Housing: Individually in suspended stainless steel wire-mesh cages.
Feed: Purina Mills Rodent Lab Chow 5001-4 in "etts" form, *ad libitum*
Water: Tap water, *ad libitum*

Vehicle

Corn oil.

Dose Preparation.

No specific details were provided. The test material was apparently mixed with the corn oil. Page 23 of the study report states that the stability of tribufos in the vehicle was established and there was no loss in 8 days (samples tested were reported to be 0.4 and 30 mg/mL). Although no actual data were presented, it was stated that the results of chemical analysis indicated that the analytically confirmed doses were 0, 2.0, 18.9 and 102.5 mg/kg since the stock samples were 95% to 102% of the nominal concentrations.

Experimental Design

The experimental design with respect to allotment of the test animals is shown in Table 1.

Table 1. Experimental Design and Allotment of Test Animals.

	Behavior Perfusion	ChE	Ma	Behavior Perfusion	ChE	Females
Control	12 6	6		12 6	6	
	12 6	6		12 6	6	
	12 6	6		12 6	6	
	12 6	6		12 6	6	

Behavior - FOB and motor activity were assessed at pretest, the time of peak effect (more specifically 6 -6.5 hours for the FOB assessments and 7 to 8.5 hours for the motor activity postdosing on day 0, see below), and on day 7 and day 14 postdosing. Cholinesterase was assessed at pretest (except brain) and at the time of peak effect (including brain). The rats were sacrificed 15 days after dosing or after motor activity assessment and perfused and assessed histopathologically for neuropathological effects.

Basis for Dose and the Time to Peak Effect

Page 18 of the study report describes the results of preliminary dose range finding studies where rats were dosed with 0, 20, 50 or 150 mg/kg tribufos (in corn oil). Observations (“detailed clinical examination”) were made at 0.5, 1, 2, 3, 3.5, 4, 24 and 48 hours for the first group dosed with 150 mg/kg only. A second group dosed with 20, 50 or 150 mg/kg was evaluated at 2,3,4, 5 and 6 hours following treatment. Clinical signs consisted of diarrhea, decreased activity, locomotor incoordination, red oral staining, urine stain, brown perianal stain. The effects were said to start at about two hours and in some cases lasted for 48 hours. The time to peak effect was said to be at 6 hours but no data or criteria for the selection of this time interval were presented. The effects were said to be present in the group dosed with 150 mg/kg but the groups dosed with 20 and 50 mg/kg were said to have only slight or no evidence of toxicity.

Additional groups of rats were dosed at 0, 2, 5 or 10 mg/kg plus the doses above for assessment of cholinesterase at 6 hours after dosing. The data were presented as percent of the control only (i.e. no data in IU/mL). Plasma ChE inhibition was statistically significant at 10 mg/kg (44% _ and 65% _) and above and there was 23% _ and 43% _ at 5 mg/kg that was not statistically significant. RBC AChE inhibition was statistically significant at 20 mg/kg (44% _ and 34% _) and above. Brain AChE was not

statistically significantly inhibited in males but at 150 mg/kg it was 15% inhibited in females.

Based on these data, it appears that the choice of the dose levels of 0, 2, 20 and 100 mg/kg were appropriate. The selection of 6 hours as the time for peak effect is not considered to be fully documented but there is no justification or indication that some other time would be more appropriate. Based on experience with other organophosphates cholinesterase inhibitors the selection of 6 hours is considered to be reasonable.

Statistics Statistical evaluations were said to be made using software from either INSTEM Computer Systems or SAS. The level to establish statistical difference was 0.05 except for Bartlett's test when it was 0.001. The following statistical tests were used.

Statistical Test	Parameters Investigated
Analysis of Variance (ANOVA) followed by Dunnett's test if a significant F-value was determined by ANOVA.	Continuous data
Bartlett's test for homogeneity of variance among groups. Homogeneous data were further analyzed by ANOVA followed by Dunnett's test for pair wise comparisons. Non-homogeneous data were assessed by the non-parametric Kruskal-Wallis Test followed by the Mann-Whitney U test for pair-wise comparisons. (See page 367)	Cholinesterase data
Repeated measured ANOVA, followed by a one way ANOVA if there was a significant interaction between dose groups and test week. Followed by Dunnett's test to determine which groups (if any) were significantly different from the control group.	FOB continuous data. Motor activity - activity for the entire session and activity for each 10 minute interval)
Two way repeated measures ANOVA using both the test interval and test occasion as the repeated measures followed by repeated measures ANOVA to determine which weeks there was a significant treatment by interval interaction. For those weeks when there was a difference, data from each interval were subjected to a one-way ANOVA and if there was a significant impact the data were further analyzed by Dunnett's test.	Motor activity - activity for interval data
General Linear Modeling (GLM) and Categorical Modeling (CATMOD) with post hoc comparisons using Dunnett's test and Analysis of Contrasts.	Categorical data from the FOB
Chi-Square Test followed by a one-tailed Fisher's Exact Test with a probability value of $p \leq 0.05$.	Pathology Data

Positive Control Data Base

References were provided that indicated that the methods and procedures were previously validated with the positive controls triadimefon and chlorpromazine. These studies have

previously been reviewed by HED. These studies are in MRID Nos.: 43656301 and 42770301.

Specific Methods and Results

1. **Mortality**

Two high dose females died and their death was attributed to treatment with tribufos. The deaths occurred within 2 to 4 days following treatment suggesting some delayed effects. Data on page 153 for rat WF3102_c which is indicated as being dead on day 6, indicate that this rat showed signs such as cool to touch (days 2-5), lacrimation (days 2, 4-5), diarrhea (days 4-5), urine stain (days 2-5), decreased activity (days 2-5), tremors (day 3) and was moribund (day 5).

Data on page 154 for rat WF3106_a which is indicated as being dead on day 5, indicate that this rat was cool to touch (days 3-4), had urine stains (days 3-4) and decreased activity (days 3-4). The immediate cause of death was not indicated for either rat.

2. **Clinical Signs** Cage side observations and detailed physical examinations were said to be conducted at least once daily¹. The compound related signs based on the individual animal report in Appendix III of the study report page 145 to 155 are shown in Table 2 (below).

Table 2 indicates that especially in females, the effects persist for as long as up to 4 days for some clinical signs. This persistent effect raises questions about whether or not the time to maximal effect which was 6 hours was appropriate for this chemical. The fact that two high dose female rats died on days 2 to 4 indicates that there may be delayed responses to tribufos.

¹Note: There appears to be an error in Table 1 of the study report on page 34. The footnote states that the observations were performed once weekly. This is inconsistent with an acute neurotoxicity study and the frequency of the clinical signs as stated on page 20 of the report. There also appeared to be some differences in the data in Table 1 (summary) of the study report and the information in APPENDIX III (individual animal response data) especially with regard to the duration of the effects. Thus, Table 2 for this DER was prepared based on the information in APPENDIX III.

Table 2. Clinical signs at 20 or 100 mg/kg* tribufos in males and females

Parameter	Males	Females
Deaths	--	2 on days 3 and 4
Decreased Activity	5 rats on days 0 to 1.	9 rats on days 1 to 3.
Perianal stain	20 mg/kg: 1 rat on day 1. 100 mg/kg: 2 rats on days 1 to 2.	20 mg/kg: 1 rat on days 1 to 2. 100 mg/kg : 4 rats on days 1 to 3
Oral Stain	1 rat on day 0	--
Salivation	1 rat at day 0	--
Urine stain	2 rats at day 0-1 or 0-2.	20 mg/kg: 3 rats on days 0 to 2 100 mg/kg: 7 rats on days 0 to 4
Cool to touch	--	6 rats on days 0 to 3
Lacrimation clear or stain (red)	--	3 rats on days 0 to 3
Diarrhea	--	2 rats on days 2 to 3
Tremors	--	1 rat on day 1
Ataxia	--	3 rats on days 0 or 2
Dehydrated	--	1 rat on day 2
Nasal stain (red)	--	1 rat on day 1

*These signs were not reported to be in the control and 2 mg/kg groups. Incidents are for the 100 mg/kg dose groups unless indicated otherwise. Data are from Appendix III pages 145 to 155 (and *not* the summary table on page 34) and refer to the "nominal day". For males, the "nominal day" 0 is the first day of dosing. For females, the first day of dosing appears to be "nominal" 2.

3. **Body Weight and Food Consumption** The rats were weighed weekly. No food or water consumption data were tabulated.

There was no separate table on body weight presented. Summary (mean \pm the standard deviation) body weight data were presented in Table 3 (pages 107 to 114) together with the grip strength and foot splay data. The high dose male group (223 \pm 15 gms) was essentially similar to the controls (224 \pm 14 gms) at pretest. At days 1, 7 and 14 the high dose male group was 2%, 6% and 3% less than the controls but statistical significance was not reported. The high dose females were equal at pretest (164 \pm 13 or 14) and showed only 3 to 4% reduction at days 7 and 14. Overall, there is at best a slight reduction in body weight in the high dose group.

4. **Functional Observational Battery**

Page 44 (data table for the males at the time to peak effect) provides evidence that the following parameters were investigated for during the FOB assessment.

<u>Home cage observations:</u> posture piloerection involuntary motor (clonic) involuntary motor tonic repetitive chewing limbs rigid and extended muscle fasciculations Opisthocomus tremors emprosthotonus myoclonic jerks convulsions explosive jumping convulsions wet dog shakes writhing gait abnormalities Incoordination, hindlimbs dragging, hindfeet pointing outward, forelimbs dragging, walking on tiptoes stilted, body dragging or flattened vocalizations other decreased activity, nutation increased reactivity	
<u>Observations During Handling</u> Easy of removal, reaction to handling, muscle tone, palpebral closure, lacrimation, salivation, nasal discharge, stains (several types and areas), other (alopecia, bite marks, broken teeth or malocclusion, dehydration, emaciation, exophthalmia, missing toe nail, cool to touch, "red zone on left shoulder").	<u>Open Field Observations</u> Same as for home cage. plus: Stereotypy (circling, nutation, head weaving, pacing, stereotypic grooming) Bizarre behavior (flopping, retropulsion, self-mutilation, tail erect, writhing) Arousal, Rearing, defecation (diarrhea, partially formed), urination, Reflex/Physiological observations: approach response, touch response, auditory response, tail pinch response, pupil size, pupil response, righting reflex, Body temperature.

Table 3 depicts the responses noted as reactions to tribufos in this study.

Based on FOB observations alone, it would appear that a systemic NOAEL and LOAEL would be 20 and 100 mg/kg. The most significant observation (reviewer's opinion) was the decrease in body temperature at 100 mg/kg. The decrease in rearing in both sexes as well as one rat in the mid dose group having minimal movement indicates that tribufos was decreasing the motor activity of the rats. This is better investigated in the following section on motor activity.

Table 3. Reactions to tribufos in the FOB assessment at the time of peak effect (6 hours postdosing).

Parameter	Males			Females		
	Control 100	2 100	20	Control 100	2 100	20
Body Temperature Cool to touch	37.3±0.3 35.4±1.4 ↓ None reported	37.2±0.3	37.1±0.4	37.6±0.4 34.8±2.9 ↓ 0 1	37.5±0.6 0	37.6±0.3 0
Decreased activity	0	0	0	0	0	0
Abnormal Posture (lying flattened)	0	2 ↑	0	0	4 ↑	0
Rearing		1			3 ↑	
Uncoordinated RR						
Arousal (normal)	0.7±1.4	1.2±1.9	1.1±1.2	2.3±2	2.7±2.8	3.0±2.7
Inactive	0.0±0.0			0.8±1.1		
Minimal movement	0	0	0	0	0	0
Some Exploratory Beh.	2	4	1	1	5	8
	0	0	0	2 ↓		
	0	1		0	0	0
	0	0	1	3 ↑		
	10	8	10	0	0	0
		10		1	7	4
				3	6	
Clear lacrimation	0	0	0	0	0	0
Clear oral stain	0	1	0	1		
		1		all 0		
Auditory stimulus ¹	0	0	0	0	0	1
Tail pinch response ¹		1		0	1	1
Pupil size (dilated)		all 0		0	0	0
		all normal		0	0	0
				1		

There were 12 animals assessed per sex for each dose group.

1. Slight response as opposed to no reaction.

There were no indications of persistent behavioral changes noted at the day 7 or day 14 FOB assessments. Table 2 above indicated that some of the clinical signs persisted for several days in females. However, there was no indication in the day 7 FOB assessment that the rats were still affected.

5. Motor Activity

Motor and locomotor activity were evaluated in 90-minute sessions with 10 minute intervals. Motor activity was measured as the number of beam interruptions that occurred during the test session. Locomotor activity was measured by eliminating consecutive counts for a given beam. Habituation was also evaluated as the decrement in activity during the test session. Table 4 illustrates that effects of tribufos on motor and locomotor activity in this study.

Both motor (interval range 40 to 81%, total 44%) and locomotor (interval range 38 to 82%, total 39%) activity were shown to be decreased at 20 mg/kg for males during intervals 1-4.

Progressively higher decreases were noted at the 100 mg/kg dose group. In females, decreases were noted in both motor (interval range 45 to 68%, total 59%) and locomotor (interval range 51 to 81%, total 68%) activity at 100 mg/kg but statistical significance was reported only for the total decrease for locomotor activity. The females had low activity with high standard deviations and thus there was no statistical difference noted for motor activity even though it could be shown that the means were much lower than the controls. It is noted that the clinical signs also indicated that the rats had decreased activity.

Table 3. Effects of tribufos on motor activity at Day 0 or the time of peak effect.

Interval	Males				Females			
	Control	2 mg/kg	20 mg/kg	100 mg/kg	Control	2 mg/kg	20 mg/kg	100 mg/kg
1 Motor	9	10	76	43	10	96	27	39
Locomotor	4±19	4±50	±24 19%↓	±27* 54	0±26	±22	10%↓ 62	%↓ 45
	6	72	52	%↓ 28	67	66	±20	33
	5±17	±32	±20 20%↓	±20* 57	±21	±13		±26 51
				%↓				%↓
2 Motor	8	71	48	25	73	80	58	29
Locomotor	0±29	±20	±18* 40	±23* 69	±26	±17	±17 215↓	±29 60
	4	46	%↓ 29	%↓ 15	45	47	36	%↓ 15
	7±23	±10	±12* 38	±14* 68	±17	±9	±10 20%↓	±16 67
			%↓	%↓				%↓
3 Motor	5	35	16	88	54	55	58	20
Locomotor	1±36	±21	±18* 69	%↓ 88	±22	±30	±17	±29 63

	2 5±23	21 ±16	%↓ 10 ±14 60 %↓	3± 7* 88 %↓	32 ±17	25 ±14	27 ±10	%↓ 6± 9 81 %↓
4 Motor	3 1±31	25 ±27	6± 12*	4± 11*	41 ±33	30 ±30	28 ±20	13 ±16
Locomotor	1 7±19	14 ±16	%↓ 3± 6* 82 %↓	%↓ 3± 6* 82 %↓	21 ±15	12 ±14	32%↓ 14 ±11	68 %↓ 4± 5 81 %↓
Total	3 13±12	30 0±181	17 6±92*	98 ±79*	32 1±142	30 5±96	29 6±63	13 1±109*
Motor	1 79±81	18 8±108	%↓ 10 9±63 39 %	%↓ 51 ±40 72 %	19 2±74	16 9±39	16 6±41 14 %↓	59 %↓ 62 ±53* 68 %↓
<u>Motor activity:</u> Intervals 5, 6 and 7 show similar reduced activity but no statistical difference (too large standard deviations). Intervals 8 and 9 do not show an effect of decreased activity. <u>Locomotor activity:</u> Intervals 5 and 6 showed a similar pattern of reduced activity but no statistical significance. Intervals 7, 8 and 9 did not indicate an effect of treatment.					<u>Motor activity:</u> Intervals 5, 6, 7, 8 and 9 did not show statistical differences although the 100 mg/kg group had the lowest activity. <u>Locomotor activity:</u> Intervals 5, 6, 7, 8 and 9 had very little activity but the high dose group was always lowest or nearly so.			

Motor activity data are from page 121 for males and 125 for females. Locomotor activity data are from page 129 for males and 133 for females.

Conclusion (FOB and motor activity). A NOAEL and LOAEL of 2 and 20 mg/kg is supported based on the decrease in motor activity at 20 mg/kg.

Cholinesterase Assessment Blood was withdrawn from the orbital plexus from the six/sex/group assigned to the satellite group two weeks (approximately prior to treatment). At approximately 6 hours after the dose administration samples of blood were again taken and the rat was sacrificed and its brain removed for cholinesterase assessment. There were no details on the exact methods used for the preparation of the blood or brain for cholinesterase assessment. The method used for assessment of cholinesterase was requested and provided by the registrant.

The SOPs indicate that plasma and RBC cholinesterase was assessed using a modified Ellman method and the Cobas Mira Plus automated instrument using acetylthiocholine iodide as the substrate.

Table 4. Plasma, RBC and brain cholinesterase at 6 hours post dosing with tribufos.

Source	Males (Day 1)			Females (Day 3)		
	Control 100	2 20		Control 100	2 20	
Plasma	0.44 (84%)* ±0.06	0.44 ±0.05	(64%)*	1.80 (98%)* ±0.62 Pretest data: 1.19 1.11 ±0.45 ±0.21	1.13 (37% ns) ±0.38 0.93 (22% ns) ±0.27	(92%)* 1.05 ±0.32
RBC	1.04 (81%)* ±0.24	0.94 (10%ns) ±0.22	(41%)*	1.17 (85%)* ±0.18	1.12 (4%) ±0.12	(55%)*
Brain	13.6 13.1 ±0.4 ±0.8	13.9 ±0.3	13.8 ±0.6	13.5 11.3 (16%)* ±0.5 ±1.1	13.9 ±0.8	13.1 ±0.5

Data are from pages 418 to 433 and are in IU/mL for plasma or RBC and IU/gm for brain. Data are ± the standard deviation or the percent inhibition for 6 animals for each group. Since the effects at 30 and 100 mg/kg were so dramatic, the standard deviations are not included for clarity of the table. Only the percent inhibition is given when there is more than a 50% decrease and the data were statistically significant.

Plasma ChE. Plasma ChE at 6 hours had standard deviations of ± 14% for males and ± 34% for females in the control group. The females showed much variation also on day -13 with the mean being 1.19 ± 0.45 (IU/mL) or ± 38%. Inspection of the individual animal data on page 426 for day -13 and on page 430 did not indicate that there was a single animal that could be considered an outlier that was causing the large standard deviation.

In males there was statistically significant inhibition at 20 mg/kg and above. In females there appears to be 37% less activity at 2 mg/kg but this was not statistically significant. At day -13 the female low dose group was already 22% less than the control (see table 4 above). An attempt was made to determine if there was an effect at 2 mg/kg in the females by inspection of the individual animal data for the six females for the control and the six females in the 2 mg/kg dose group at day minus 13 and at the day of dosing (Day 1). This analysis is shown as follows.

Control			2		
			mg/kg		
Animal	Day -13	Day 1	Animal	Day -13	

Day 1						
301	1.24		2.06 (66%)	1301	0.64	0.80 (25%)
302	0.71		1.52 (114%)	1302	0.80	1.29 (61%)
303	1.16		1.50 (29%)	1301	0.73	0.70 (-4%)
304	1.39		1.93 (39%)	1304	0.91	1.08 (19%)
305	0.71		0.97 (37%)	1305	1.29	1.15 (-11%)
306	1.90		2.80 (47%)	1306	1.23	1.77 (44%)
Mean	1.19±0.45.	1.80±0.62		0.93±0.27	1.13±0.38	

Day 1 vs Day -13: Within control: $(1.80/1.19 \times 100 = +51\%)$
Within 2 mg/kg $(1.13/0.93 \times 100 = +22\%)$

Day -13 control vs 2 mg/kg $(1.19/0.93 \times 100 = 22\% \downarrow)$

Day 1 control vs 2 mg/kg $(1.13/1.80 \times 100 = 37\% \downarrow)$

This analysis shows that there is an increase in activity of 51% between days -13 and day 3 for the control group but only 22% for the 2 mg/kg dose group. Some of the 2 mg/kg dose group actually have negative values (i.e. rats 303 and 305). This observation would support the conclusion that there is in fact inhibition at 2 mg/kg.

The comparison of the control and 2 mg/kg dose group in females is considered confounded by the variability of the data especially in the control group. The fact that the 2 mg/kg rather consistently gave lower values when day 3 was compared with the pretest day -13 for each animal than did the control group suggests that there is inhibition at the 2 mg/kg dose group. Overall, these data suggest that the low dose group is inhibited as much as 15% relative to the control (i.e. 37% noted at day 3 minus 22% to account for the difference in the pretest assessment). This would be supported by the observation that there is already 92% inhibition at 20 mg/kg the next higher dose in females.

RBC AChE. RBC AChE had standard deviations of $\pm 23\%$ in males and $\pm 15\%$ in females in the control groups. At day -14, the males also had standard deviations of $\pm 26\%$. There was thus poor precision in the assessment of RBC AChE. See Table 4 above.

Both males and females have statistically significant inhibition of RBC AChE at 20 and 100 mg/kg. The 10% apparent decrease in activity at 2 mg/kg in males is not considered to be biologically meaningful especially since there is more plasma ChE inhibition (64%) at 20 mg/kg than RBC AChE inhibition (41%) at 20 mg/kg.

Brain AChE. Brain AChE had standard deviations of only 3-4% meaning very good precision for the data. Brain AChE was inhibited (16%) only in females and this is consistent with the dose range finding study data.

Conclusion (cholinesterase data). The NOAEL is 2 mg/kg and the LOAEL is 20 mg/kg with

both plasma and RBC cholinesterase being inhibited at this dose level. The dose level of 2 mg/kg may actually be a threshold with there being as much as 15% inhibition in females. RRBIII does not consider that 15% inhibition at 2 mg/kg is sufficient to justify including this dose level as the LOAEL for inhibition of plasma cholinesterase especially when there is so much variability in the control data. Plasma ChE is more susceptible than RBC AChE and females seem more susceptible than males. Brain AChE becomes inhibited only at 100 mg/kg and in females only.

7. **Pathology**

A separate pathology report prepared by B.P. Stuart was presented in Appendix IX of the study report which addressed the necropsy, brain weight and histopathology as well as contained some tables on cholinesterase assessment.

Gross Pathology. There were no effects of treatment as indicated by gross necropsy and brain weight and relative brain weight was not affected (refer to Tables 385 on page of the study report).

Brain weight. The mean brain weights for the control and high dose males were 1.869 ± 0.049 and 1.871 ± 0.097 gms and for females were 1.747 ± 0.064 and 1.788 ± 0.091 gms indicating no difference. Since there was also no difference in mean body weight, there was thus also no difference in the brain to body weight ratio.

Histopathology. Histopathology was assessed for the 6/sex for the control and high dose group only. The tables on page 404 to 407 indicate that eight levels of the brain (level 1, olfactory bulbs; levels 2-6 - cerebral cortex; levels 2-5 - caudate-putamen/globus pallidus; levels 5-6 hippocampus; levels 4-5 - thalamus and hypothalamus; level 6 - midbrain including tectum, tegmentum and cerebral peduncle; level 7-8 - cerebellum and medulla oblongata. The eyes, the dorsal root and gasserian ganglion, gastrocnemius muscle, left and right sural nerve, left and right sciatic nerve, left and right tibial nerve, optic nerves, spinal cord (cauda quina, cervical, lumbar, thoracic and roots) were all examined for six males and females for the control and 100 mg/kg dose groups. Prior to histopathological examination, the rats were prepared by perfusion with aldehyde fixative following prior flushing with 0.7% sodium nitrite in phosphate buffer and a solution of 10% formalin. The brains were reported to be trimmed in a standard manner using metal rodent brain matrix (RBM-4000C, Activated Systems, Inc., Warren, MI).

Brain and spinal cord sections were reported to be embedded in paraffin and sectioned at approximately 5 μ m. Three sections were collected from each level and stained with hematoxylin and eosin (H&E), Luxol fast blue-cresyl violet (LFB/CV) and Sevier -Munger silver stains respectively. The peripheral nerves and eyes and muscle were reported to be embedded in glycol methacrylate (GMA), sectioned at 2 to 3 μ m and stained with a modified Lee's stain. Cross and longitudinal sections of the peripheral nerves were prepared.

Inspection of the pathology data indicated occasional incidence of degeneration of nerve fibers but these were in the control group as well as those dosed with 100 mg/kg. Table 5

summarizes the incidents and severity of the nerve degeneration data.

Table 5. Net incidence and severity of nerve degeneration in rats dosed with tribufos.

	Males	Females
	Control 100 mg/kg	Control 100 mg/kg
Total incidence ¹	11 7	17 12
Distribution		
-Brain Level 7	0	0
-Eyes (retinal)	0	1 (1.0)
-Dorsal root gang.	0	1 (1.0)
-Sural nerve (l/r)	0	0
-Sciatic nerve (r)	2 (1.0)	3 (1.0)
-Tibial nerve	2 (1.0)	1 (1.0)
-Spinal cord	2 (1.0)	1 (1.0)
cauda equina	1 (1.0)	2 (1.0)
cervical	0	2 (1.5)
lumbar	1 (1.0)	1 (1.0)
thoracic	4 (1.0)	0
	1 (1.0)	2 (1.5)
	0	0
	0	1 (1.0)
	0	3 (1.0)
	0	2 (1.0)
	0	3 (1.0)
	0	0
	3 (1.3)	4 (1.0)
	2 (1.0)	2 (1.0)
Severity	1.0 to 1.3 1.0	1.0 to 1.5 to 1.5 1.0
Rats Affected	6 5	6 5

Data are from Table MP 1-SUM page 404 to 407. Data are number of rats affected and the severity in (). Note inspection of the individual animal pathology reports indicates that nearly all of these were focal. Multifocal was noted only occasionally and in both control and high dose groups.

Since there were more total effects noted in the control groups for both males and females and since there was no difference in the severity, it is concluded that there were no histopathological effects in the nervous system due to tribufos treatment in this study.

Conclusions and Discussion

Classification.

This study is classified as ACCEPTABLE/Guidelines and satisfies the requirement for a series 870.6300 acute neurotoxicity study in rats for tribufos.

Study Deficiencies/Comments. There were no study deficiencies that were considered to compromise the interpretation of the data. The following comments are being made.

1. HED is in the process of reevaluating the positive control data base for *all* laboratories conducting acute, subchronic and developmental neurotoxicity studies. The positive control data base from the Bayer Corporation in Stilwell, Kansas was reviewed several years ago and *may* need updating to assure that all personnel currently conducting studies have demonstrated their skills in conducting neurotoxicity studies.
2. There is an inconsistency in the text, summary tables and the individual animal data tables with regard to assigning the day of the study. In some places, particularly in the text, the first day of dosing is day 0. In the tables on individual animal data, the day is referred to as the “nominal day) and is not the same numerically. In particular, the text and tables say that two female rats died from days 2 and 4. However, the individual animal data tables indicate that these animals died on “nominal day” 5 or 6.
3. The observation that there were deaths on days 2 to 4 and that some effects were said to have started after the 6 hour interval raises further questions about the persistence and possible delayed effects of tribufos. Since the preliminary studies indicated that the effects started at about 2 hours and it was stated that the more obvious signs had a peak of effect at 6 hours (probably based on locomotor activity), the study can be considered to meet the criteria for assessing at the time of peak effects. Conducting additional studies at higher doses to determine if there are delayed effects that may be occurring between the time of peak effect and the day 7 FOB/motor activity assessment and attempting to explain why the two females died at a time well after the time of peak effects would be of interest but are not required since the study has demonstrated the required NOAEL and LOAEL's for cholinesterase inhibition and FOB and motor activity responses and there was no histopathology persisting to study termination.